



## Anti-metastatic mechanism of Tian-Xian Liquid (TXL) and its bioactive fractions in human colorectal cancer cells and xenograft models

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Colorectal carcinoma is the second most prevalent cancer with an up-rising trend in Hong Kong (Hong Kong Cancer Registry). Traditional Chinese medicine acts as a complementary alternative for tumour therapy with minimal side-effects and traumatic injuries. Tian-Xian Liquid (TXL), one of the well-known natural medicinal herbal formulations, has been commercially used as an anticancer dietary supplement for a decade without known adverse effects. This study aimed to investigate the anti-metastatic property of TXL and its bioactive fractions [butanol fraction (BU), ethyl-acetate fraction (EA) and aqueous fraction (WA)] at molecular level on human colorectal cancer in vitro (HT-29 cancer cells) and in vivo (nude mice xenografts). For the cell model, TXL and its bioactive fractions have similar anti-proliferative effects by MTT assay. At 4-hour-incubation, IC50 values were obtained at 1% (V/V) TXL, 1.25% (V/V) BU, 5% (V/V) EA and 0.3125% (V/V) WA. At IC50, TXL and its bioactive fractions significantly reduced the MMP2 and MMP7 expressions at mRNA level by real-time PCR. At protein level, TXL, BU and EA correspondingly down-regulated MMP2 (active form) and MMP7 protein from 24 to 48 hours; TXL and BU also down-regulated VEGF protein expression; however, no such effect was found in WA-treated cells. Further, only TXL, EA and WA effectively inhibited the cell migration at 48 hours incubation by woundhealing assay. For the xenografts models, MMP2 and MMP7 mRNA expressions were reduced by TXL-, BU- and EA-treated xenografts; however no effects on MMP2 protein expression in all drug-treated xenografts. The VEGF protein expression was significantly down-regulated in TXL- and WA-treated xenografts. Further, TXL, BU and WA effectively inhibited the tumor growth without altering the body weight of the xenografts. In summary, the Chinese medicinal formulation, TXL, demonstrated the most effective anti-metastatic ability on human colorectal cancer in vitro and in vivo.

## Trans-differentiation of breast cancer by GATA3 reduces primary growth and metastasis

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Low-grade breast cancer is associated with increased differentiation and reduced metastases, suggesting that reprogramming tumor cells to a more differentiated state could improve outcome. Previous differentiation strategies for breast cancer have failed. Utilizing a novel differentiation therapy approach, we have reprogrammed aggressive, basal, triple-negative Breast Cancer (BrCa) (generally with poor prognosis) towards a less aggressive phenotype by manipulating expression of the key mammary luminal differentiation transcription factor, GATA3. GATA3 is essential for programming undifferentiated mammary cells into a luminal subtype while myoepithelial/basal cells fail to express GATA3. Significantly, GATA3 expression is highly correlated with the luminal, more differentiated BrCa phenotype. We hypothesized that ectopic expression of GATA3 in metastatic, basal BrCa cell lines will reprogram them to a more differentiated, less metastatic phenotype. Over-expressing GATA3 in human basaltypic MDA-MB-231 (231-GATA3) BrCa cells induced significant morphological changes in 2- and 3-D cultures compared to control cells (231-Empty). 231-Empty cells maintained a spindle, elongated morphology, while 231-GATA3 cells became rounded and larger. In 3-D Cultrex, 231-GATA3 cells appeared smaller, more organized, and rounded compared to 231-Empty cells. Microarray profiling of 231-GATA3 vs. 231-Empty cells revealed gene expression changes associated with increased adhesion, reduced extracellular matrix remodeling factors and reduced metastasis. Western blot confirmed re-expression of E-cadherin and reduction of fibronectin in 231-GATA3 cells, indicative of a more luminal phenotype. The gene most downregulated by GATA3 was Lysyl Oxidase (LOX) and confirmed by qPCR. LOX has been shown to be critical for metastatic progression through matrix remodeling. Knock down of GATA3 in the GATA3 positive BT474 cell line increased LOX expression. Retrospective analysis of microarray profiles of BrCa cells revealed an association of LOX with the basal subtype and a statistical inverse association between LOX and GATA3. SCID mice injected with 231-GATA3 cells by tail vein showed significantly fewer metastatic lesions in the lung compared to control cells ( $p < 0.05$ ). Importantly, restoration of LOX in 231-GATA3 cells by lentiviral transduction reversed the reduced metastatic phenotype. This demonstrates that expression of GATA3 reprogrammed a poorly differentiated basal cell towards a less metastatic phenotype and the GATA3 dependent LOX down-regulation is responsible for the reduced observed metastasis. These findings suggest that transcription factor-induced differentiation pathways may be potentially novel therapeutic molecular targets to inhibit metastatic disease progression in combination with standard therapeutic treatments.